

WHAT IS CLAIMED IS:

1. A method of performing a primer extension reaction, comprising:
 - obtaining an amplicon having a sequence generated from a target nucleic acid
 - 5 and a sequence generated from a first strand amplification primer, by amplifying a target nucleic acid having a variant nucleotide flanked by an invariant nucleotide, wherein a first strand amplification primer is employed that comprises a 5' tag substantially incapable of hybridizing to the target nucleic acid under amplification conditions, and wherein the 5' tag contains the variant nucleotide of the target nucleic
 - 10 acid, and employing a second strand amplification primer;
 - employing the amplicon in a primer extension reaction wherein the identity of the variant nucleotide in the sequence generated from the target nucleic acid is determined by hybridizing a first identification primer immediately adjacent to the variant nucleotide in the sequence generated from the target nucleic acid;
 - 15 hybridizing a second identification primer immediately adjacent to the variant nucleotide in the sequence generated from the amplification primers;
 - extending the first and the second identification primers in the presence of one or more nucleotides and a polymerizing agent;
 - determining the identity of the variant nucleotide generated from the target
 - 20 nucleic acid; and
 - comparing extension product of the first identification primer and extension product of the second identification primer, thereby performing the primer extension reaction.
- 25 2. A method according to claim 1, wherein immediately adjacent in the 5' direction to the variant nucleotide in the 5'tag is the invariant nucleotide to the 5' direction of the variant nucleotide of the target nucleic acid.
3. A method according to claim 1, wherein immediately adjacent in the 3'
- 30 direction to the variant nucleotide in the 5'tag is the invariant nucleotide to the 3' direction of the variant nucleotide of the target nucleic acid.
4. A method according to claim 1, wherein immediately adjacent in the 3' direction to the variant nucleotide in the 5'tag is the invariant nucleotide to the 3'

direction of the variant nucleotide of the target nucleic acid, and immediately adjacent in the 5' direction to the variant nucleotide in the 5' tag is the invariant nucleotide to the 5' direction of the variant nucleotide of the target nucleic acid.

5 5. A method according to claim 1, wherein the 5' tag has at least two invariant nucleotides immediately adjacent in the 3' direction to the variant nucleotide, and at least two invariant nucleotides immediately adjacent in the 5' direction to the variant nucleotide, and wherein the at least two invariant nucleotides immediately adjacent in the 3' direction and the at least two invariant nucleotides immediately adjacent in the
10 5' direction are selected so as to be substantially homologous to the corresponding nucleotides flanking the variant nucleotide in the target nucleic acid.

6. A method according to claim 1, wherein the first and the second identification primers bear a detectable characteristic.

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7. A method according to claim 6, wherein the detectable characteristic on the first identification primer is different from the detectable characteristic on the second identification primer.

20 8. A method according to claim 1, wherein the identity of the variant nucleotide in the 5' tag is varied so as to generate a population of amplicons in which the identity of the variant nucleotide is fixed at a known ratio.

9. A method according to claim 8, wherein the identity of the variant nucleotide in
25 the 5' tag is varied so as to generate a population of amplicons that is a balanced heterozygous population with respect to the variant nucleotide.

10. A method according to claim 1, wherein the target nucleic acid comprises nucleic acids from two or more individuals.

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11. A method according to claim 1, wherein two or more variant nucleotides are identified.

12. A method according to claim 11 wherein the two or more variant nucleotides are on the same nucleic acid molecule.

13. A method according to claim 11, wherein the two or more variant nucleotides
5 are on different nucleic acid molecules.

14. A method according to claim 1, wherein the identification primers are extended by one or more labeled nucleotide bases, and are capable of being detected by a characteristic selected from the group consisting of mass, apparent mass, molecular
10 weight, apparent molecular weight, a combination or ratio of mass and charge, number of bases, magnetic resonance, spectrophotometry, fluorometry, electric charge, polarimetry, light scattering, luminescence and antigen-antibody interaction.

15. A method according to claim 1, wherein the identification primers are extended
15 by a chain terminator.

16. A method according to claim 14, wherein the chain terminator is a dideoxynucleotide or an acyclo terminator.

20 17. A method according to claim 14, wherein the chain terminator is labeled with a detectable moiety.

18. A method according to claim 14, wherein the identification primers comprise a tag capture moiety.
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19. A method according to claim 18, wherein the identification primers are captured on an array.

20. A method according to claim 19, wherein the array is an addressable array.
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21. A method according to claim 19, wherein the array is a virtual array.

22. A method of performing a primer extension reaction, comprising:

obtaining a sample comprising target nucleic acid from one or more individuals;

obtaining an amplicon population having a sequence generated from the sample and a sequence generated from a tagged first strand amplification primer, by
5 amplifying nucleic acids in the sample having a variant nucleotide that is a transversion flanked in the 5' direction by an invariant nucleotide and flanked in the 3' direction by an invariant nucleotide, wherein the tagged first strand primer is employed that comprises a 5' tag substantially incapable of hybridizing to target nucleic acids in the sample, and wherein the 5' tag contains the variant nucleotide
10 with its flanking invariant nucleotides, and wherein a second strand amplification primer is employed;

employing the amplicon population in a primer extension reaction wherein the identity of the variant nucleotide in the sequence generated from the sample is determined by hybridizing a first identification primer immediately adjacent to the
15 variant nucleotide in the sequence generated from the sample;

hybridizing a second identification primer immediately adjacent to the variant nucleotide in the sequence generated from the amplification primer;

extending the first and the second identification primers in the presence of one or more nucleotides and a polymerizing agent;

20 determining the identity of the variant nucleotide generated from the sample;
and

comparing extension product of the first identification primer and extension product of the second identification primer, thereby performing the primer extension reaction.

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23. A method according to claim 22, wherein the flanking invariant nucleotide in the 5' direction is complementary with the flanking invariant nucleotide in the 3' direction.

30 24. A method according to claim 22, wherein the first strand amplification primer comprises the two or more nucleotides in the 5' direction immediately adjacent to the variant nucleotide of the first strand amplification primer, wherein the two or more nucleotides are identical to the two or more nucleotides immediately adjacent in the 5' direction of the variant nucleotide in the target.

25. A method according to claim 22, wherein the first strand amplification primer comprises the two or more nucleotides in the 3' direction immediately adjacent to the variant nucleotide of the first strand amplification primer, wherein the two or more
5 nucleotides are identical to the two or more nucleotides immediately adjacent in the 3' direction of the variant nucleotide in the target nucleic acid.

26. A method according to claim 22, wherein the first strand amplification primer comprises the two or more nucleotides in the 5' direction immediately adjacent to the
10 variant nucleotide of the first strand amplification primer, and the two or more nucleotides in the 3' direction immediately adjacent to the variant nucleotide of the first strand amplification primer, each arranged as to be identical to the corresponding nucleotides flanking the variant nucleotide in the target nucleic acid.

15 27. A method according to claim 23, wherein the second strand amplification primer comprises a 5' tag having the invariant nucleotide.

28. A method according to claim 27 wherein the variant nucleotide is flanked by the same complementary flanking invariant nucleotides in the target nucleic acid.
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29. A method according to claim 28, wherein the identity of the variant nucleotide in the 5' tag is varied so as to generate a population of amplicons wherein the identity of the variant nucleotide is varied at a known ratio.

25 30. A method according to claim 28, wherein the identity of the variant nucleotide in the 5' tag of the first strand amplification primer and the second strand amplification primer is varied so as to generate an amplicon population comprising a ratio of 1:1 and a ratio of 3:1 with respect to the identity of the nucleotides in the amplicon population generated by the 5' tags.

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31. A method according to claim 22, wherein the first and the second identification primers bear a detectable characteristic.

32. A method according to claim 22, wherein the detectable characteristic on the first identification primer is different from the detectable characteristic on the second identification primer.

5 33. A method according to claim 22, wherein two or more variant nucleotides are identified.

34. A method according to claim 33, wherein the two or more variant nucleotides are on the same nucleic acid molecule.

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35. A method according to claim 33, wherein the two or more variant nucleotides are on different nucleic acid molecules.

15 36. A method according to claim 22, wherein the identification primers are extended by one or more labeled nucleotide bases, and are capable of being detected by a characteristic selected from the group consisting of mass, apparent mass, molecular weight, apparent molecular weight, a combination or ratio of mass and charge, number of bases, magnetic resonance, spectrophotometry, fluorometry, electric charge, polarimetry, light scattering, luminescence and antigen-antibody
20 interaction.

37. A method according to claim 22, wherein the identification primers are extended by a chain terminator.

25 38. A method according to claim 37, wherein the chain terminator is a dideoxynucleotide or an acyclo terminator.

39. A method according to claim 37, wherein the chain terminator is labeled with a detectable moiety.

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40. A method according to claim 22, wherein the identification primers comprise a tag capture moiety.

41. A method according to claim 40, wherein the identification primers are captured on an array.

42. A method according to claim 41, wherein the array is an addressable array.

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43. A method according to claim 41, wherein the array is a virtual array.

44. A method according to claim 23, wherein the second strand amplification primer comprises a 5' tag having the same variant nucleotide, the same invariant nucleotide flanked in the 5' direction, and the same invariant nucleotide flanked in the 3' direction as the first strand amplification primer, and wherein the first strand amplification primer reflects a transversion ratio of 1:1 in the variant nucleotide and wherein the second strand amplification primer reflects a transversion ratio of 1:3 in the variant nucleotide, and wherein at least three identification primers are employed in the primer extension reaction.

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45. A method according to claim 22, wherein the individuals are sheep.

46. A method according to claim 22, wherein at least one of the one or more individuals displays at least one complex genotype.

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47. A method according to claim 45, wherein the target nucleic acid comprises the PrP locus.

48. A method of performing primer extension utilizing at least two amplification primers comprising:

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obtaining a target nucleic acid comprising a variant nucleotide flanked by an invariant nucleotide;

hybridizing to the target nucleic acid a first amplification primer having a 5' tag comprising the variant nucleotide flanked by the invariant nucleotide, wherein the 5' tag is substantially unable to hybridize to the target nucleic acid, and hybridizing a second amplification primer; and

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extending the amplification primers in the presence of at least one or more nucleotides and a polymerizing agent, thereby performing primer extension.

49. A composition, comprising

a primer having a region capable of hybridizing to a target nucleic acid wherein the target nucleic acid comprises a variant nucleotide and an invariant nucleotide, and
5 wherein the primer further comprises a 5' tag region having the variant nucleotide and the invariant nucleotide of the target nucleic acid, and wherein the 5' tag region is substantially incapable of hybridizing to the target nucleic acid under conditions suitable for amplification of the target nucleic acid.

10 50. A composition according to claim 49, wherein the target nucleic acid comprises the scrapie locus.

51. A method of monitoring the efficiency of incorporation of chain terminators into primers in a primer extension reaction, comprising:

15 generating a population of amplicons from a mixed sample of target nucleic acid, wherein the population of amplicons comprises sequences at known ratios;

performing primer extension reactions on the population of amplicons employing chain terminators and employing a population of primers specific for the sequences;

20 detecting and measuring efficiency of incorporation of chain terminators into the population of primers at the known ratios, thereby monitoring the efficiency of incorporation of chain terminators into primers in a primer extension reaction.

using the information generated on the efficiency of incorporation of chain terminators at known ratio to interpret observed efficiencies of incorporation of these
25 chain terminators into primers targeted against polymorphisms of unknown ratio.

52. A method according to claim 51, wherein efficiency of incorporation of chain terminators into the population of primers at the known ratios is employed to interpret observed efficiencies of incorporation of the chain terminators into primers targeted
30 against polymorphisms of unknown ratio.

53. A method of performing a primer extension reaction, comprising:

obtaining a sample comprising target nucleic acid from one or more individuals;

obtaining an amplicon population having a sequence generated from the sample and a sequence generated from a tagged first strand amplification primer, by amplifying nucleic acids in the sample having a variant nucleotide, wherein the tagged first strand primer is employed that comprises a 5' tag substantially incapable of hybridizing to target nucleic acids in the sample, and wherein the 5' tag contains the variant nucleotide, and wherein a second strand amplification primer is employed; employing the amplicon population in a primer extension reaction wherein the identity of the variant nucleotide in the sequence generated from the sample is determined by hybridizing a first identification primer immediately adjacent to the variant nucleotide in the sequence generated from the sample; hybridizing a second identification primer immediately adjacent to the variant nucleotide in the sequence generated from the amplification primer; extending the first and the second identification primers in the presence of one or more nucleotides and a polymerizing agent; determining the identity of the variant nucleotide generated from the sample; and comparing extension product of the first identification primer and extension product of the second identification primer, thereby performing the primer extension reaction.

54. A method according to claim 53, wherein the variant nucleotide represents a transversion.

55. A method of screening animals for susceptibility to a disease or disorder, comprising:

determining the identity of polymorphic nucleotides at three or more alleles at a locus; and

employing the identities of the polymorphic nucleotides to determine whether the animal is susceptible to a disease or disorder.

56. A method according to claim 55, wherein the animals are sheep.

57. A method according to claim 55, wherein the animals display a complex genotype with respect to at least one locus.

58. A method according to claim 55, wherein the disease or disorder is a transmissible encephalopathy.

5 59. A method according to claim 55, wherein the disease or disorder is scrapie.

60. A method of breeding scrapie-resistant sheep, comprising:

determining the identity of polymorphic nucleotides two or more alleles at the PrP locus of a male sheep and a female sheep using a method according to claim 1;

10 employing the identities of the polymorphic nucleotides to determine whether the male sheep and the female sheep possess two or more alleles that are not associated with susceptibility to scrapie; and

breeding male sheep and female sheep that possess two or more alleles that are not associated with susceptibility to scrapie.

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61. A method of breeding scrapie-resistant sheep, comprising:

determining the identity of polymorphic nucleotides two or more alleles at the PrP locus of a male sheep and a female sheep using a method according to claim 22;

20 employing the identities of the polymorphic nucleotides to determine whether the male sheep and the female sheep possess two or more alleles that are not associated with susceptibility to scrapie; and

breeding male sheep and female sheep that possess two or more alleles that are not associated with susceptibility to scrapie.

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